JOURNAL OF VIROLOGY, June 2002, p. 6408–6412 Vol. 76, No. 12
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.12.6408–6412.2002

Pre- and Postexposure Prophylaxis of Ebola Virus Infection in an Animal Model by Passive Transfer of a Neutralizing Human Antibody

Paul W. H. I. Parren,[1]† Tom W. Geisbert,[2] Toshiaki Maruyama,[1]‡ Peter B. Jahrling,[2]* and Dennis R. Burton[1]*

Departments of Immunology and Molecular Biology, The Scripps Research Institute, La Jolla, California 92037,[1] and U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702[2]

Received 12 October 2001/Accepted 8 March 2002

A neutralizing human monoclonal antibody, KZ52, protects guinea pigs from lethal Ebola Zaire virus challenge. Administration before or up to 1 h after challenge resulted in dose-dependent protection by the antibody. Interestingly, some antibody-treated animals survived despite developing high-level viremia, suggesting that the mechanism of protection by KZ52 may extend beyond reduction of viremia by virus neutralization. KZ52 is a promising candidate for immunoprophylaxis of Ebola virus infection.

Antiviral antibodies play a critical role in protection against infection or disease following viral challenge. Classically, the role of antibody has been demonstrated by transferring serum or immune globulin from an immune to a naive individual prior to or immediately following challenge with the pathogen. Infectious agents for which this approach has been successful include important human viral pathogens such as respiratory syncytial virus, hepatitis A virus, measles virus, poliovirus, and rabies virus (reviewed in references 8, 10, and 28). Generally, the protective ability of transferred immunoglobulin has been associated with neutralizing activity in vitro (28). However, for some viruses, the passive transfer approach has been difficult because natural infection appears to elicit rather poor neutralizing antibody responses (7). A prominent example is human immunodeficiency virus type 1 (HIV-1), which in many infected individuals elicits neutralizing antibody responses of rather poor quality and for which protection with passively transferred immune globulin has not been demonstrated. In the case of HIV-1, a small number of neutralizing monoclonal antibodies (MAbs) isolated from infected individuals have then been invaluable in demonstrating that antibodies can indeed provide protection against virus challenge (2, 13, 24, 25, 29).

A number of remarkable similarities exist between the humoral response to filoviruses, in particular Ebola virus, and the response to HIV-1 discussed above. In Ebola virus infection, there is also little evidence for the development of neutralizing antibodies in the sera of infected individuals. Two of the four known strains of Ebola virus, Zaire and Sudan, are responsible for the majority of infections and have been implicated in all confirmed lethalities due to Ebola virus infection (6). The pathogenesis of infection with Ebola Zaire and Ebola Sudan viruses is typically swift, and most infected subjects die before detectable antibody responses have been established. However, whereas survivors do seroconvert, neutralizing antibody titers in serum remain very low (17, 19, 26), reminiscent of the observations with HIV-1 infection. Immunoprophylaxis of Ebola virus infection, using convalescent-phase serum, has been employed, but with disputed and limited success (1, 4, 12, 27). In contrast, for another filovirus, Marburg virus, there is evidence for the presence of neutralizing antibodies in serum. Thus, guinea pigs were protected by incubating Marburg virus with serum from convalescent patients prior to challenge (ex vivo neutralization) (32), and passive transfer of serum from immunized and convalescent animals, furthermore, protected naive guinea pigs from homologous Marburg virus challenge (14).

A number of studies have attempted to demonstrate an impact of antibody on Ebola virus infection. The most elaborate studies have been performed with neutralizing equine immunoglobulin G (IgG) against Ebola virus (16, 17). The equine IgG was originally developed by a group of Russian investigators who determined that horses were not susceptible to Ebola virus infection and that sera with high neutralizing antibody titers could be obtained by immunization with liver homogenates from Ebola virus-infected monkeys (18). Guinea pigs were completely protected when the neutralizing equine IgG was given before, but not after, Ebola virus challenge (17). Similar results for guinea pigs were obtained with neutralizing ovine and caprine IgGs against Ebola virus. A concern with regard to these latter experiments, however, is that the immune sera likely contained considerable titers of antibodies against guinea pig cell antigens because they were raised against homogenates of Ebola virus-infected guinea pig liver (20). In addition, a number of studies have been performed with a mouse-adapted Ebola virus in a mouse challenge model (5). However, in contrast to Ebola virus infection in other animal models, it is relatively easy to protect mice from infec-

* Corresponding author: Mailing address for Dennis R. Burton: Department of Immunology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Phone: (858) 784-9298. Fax: (858) 784-8360. E-mail: burton@scripps.edu. Mailing address for Peter B. Jahrling: Headquarters, USAMRIID, Ft. Detrick, Frederick, MD 21702. Phone: (301) 619-2772. Fax: (301) 619-4625. E-mail: Peter.Jahrling@DET.AMEDD.ARMY.MIL.

† Present address: Genmab, Utrecht, The Netherlands.

‡ Present address: Alexion Antibody Technologies, San Diego, CA 92121.

tion with the mouse-adapted Ebola virus. For example, the mouse-adapted Ebola virus caused disease only when given intraperitoneally (i.p.), whereas virus administered intramuscularly (i.m.) or subcutaneously (s.c.) was not pathogenic and protected against subsequent i.p. challenge (5). Furthermore, poorly neutralizing antibodies were able to provide protection against challenge in this model (37).

A more stringent test of the neutralizing equine antibodies was performed in challenge experiments with cynomolgus macaques. In contrast to the guinea pig experiments, all macaques became infected, although some benefit, in the form of a slight delay in the onset of viremia, was observed (17). In other studies, the neutralizing equine IgG protected baboons from low-dose (<30 50% lethal doses [LD_{50}]) Ebola virus challenge when the IgG was given up to 1 h after infection, and high neutralization titers (1:128 to 1:512) were achieved in serum (3, 20). Neutralizing ovine serum similarly protected baboons against low-dose Ebola virus challenge (0.6 LD_{50}) (21).

We recently developed a human anti-Ebola virus MAb, IgG1 KZ52, which neutralizes the virus effectively (23). This antibody may be useful as an antiviral agent in the prophylaxis of Ebola virus infection. We now present the results of the first animal studies in which this antibody was used. We show that the antibody protects against robust Ebola virus challenge in the guinea pig model when administered up to 1 h after virus challenge.

An animal model for Ebola virus infection and pathogenesis has been developed in guinea pigs by infection of strain 13 guinea pigs with the Ebola Zaire virus (Mayinga) followed by four sequential passages of virus in naive guinea pigs, using homogenized spleens. Spleen homogenate from the final passage was used to inoculate Vero 76 cells to prepare the guinea pig-adapted Ebola virus challenge stock (11). The resulting guinea pig-adapted strain gives rise to high-level viremia in plasma (typically $>10^5$ PFU) and is highly lethal in guinea pigs, typically resulting in death between 8 and 11 days following infection (11).

We have previously described a human antibody, IgG1 KZ52, directed against the Ebola Zaire virus glycoprotein, which was found to effectively neutralize Ebola Zaire virus (1995) with a 50% inhibitory concentration (IC_{50}) of 0.3 μg/ml and an IC_{90} of 2.6 μg/ml (22, 23). In the present study, we used another Ebola Zaire virus isolate (Mayinga), which was obtained from human serum specimen 057931 by one passage in Vero 76 cells. To compare the sensitivities of the original Ebola Zaire virus (Mayinga) and the guinea-pig adapted Ebola Zaire virus strain to neutralization by IgG1 KZ52, we performed additional plaque reduction neutralization assays as described previously (23). Ebola Zaire virus (Mayinga) virus and the guinea pig-adapted virus were neutralized (IC_{50}) at 0.9 and 1.2 μg/ml, respectively. These numbers are not significantly different from each other or from the neutralization titers determined in our initial report with Ebola Zaire virus (1995). In vivo passaging of the virus, therefore, did not affect its neutralization sensitivity to this antibody.

To evaluate whether IgG1 KZ52 could protect against Ebola virus infection in an animal model, we performed passive antibody transfer experiments with guinea pigs (strain 13; weight, 400 to 450 g) followed by challenge with the guinea pig-adapted Ebola virus strain described above.

In conducting research using animals, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* (27a). The U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) animal facilities and animal care and use program are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Infectious material and animals were handled in a maximum-containment biosafety level 4 facility at USAMRIID under standard operating conditions.

IgG1 KZ52 was produced and purified as detailed by Parren et al. (29); it was >98% pure, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and contained <1 IU of endotoxin/ml, as determined in a quantitative chromagenic *Limulus* amoebecyte lysate assay (BioWhittaker, Walkersville, Md.). The half-life of IgG1 KZ52 was determined to be 7 days.

Protection against virus challenge by neutralizing antibodies in naive animals usually requires neutralizing antibody titers in serum (IC_{90}) higher than, very roughly, 1:100 (28). Whether this was also the case for challenge with Ebola virus infection was unclear. Therefore, we first performed a KZ52 titration experiment. Challenge was performed with a robust dose of the guinea pig-adapted Ebola Zaire virus (10,000 PFU s.c. in the right upper thoracic limb [11]), and antibody was injected i.p. at the time of challenge (indicated as time zero), performed by administering the antibody several minutes after injection of the virus. We started with a high dose of KZ52, 50 mg/kg of body weight, resulting in an antibody concentration in serum of approximately 500 μg/ml (neutralization antibody titers of ~1:200), and two lower doses of the antibody (5 and 0.5 mg/kg). Animals were carefully monitored for signs of disease and for survival during the study's time course of 2 months, and Ebola virus plasma viremia was determined with day 7 serum by plaque assay. The results show that dose-dependent protection was achieved (Table 1). The highest dose of KZ52 protected all animals from lethal infection, the intermediate dose provided partial protection, and the lowest dose of KZ52 did not protect the animals from disease. Animals treated with corresponding doses of a control human IgG (obtained from the serum of a healthy individual) and untreated animals all became infected and died 7 to 12 days postchallenge (except one animal in the control group, which developed only a very low plasma viremia [2.6 log PFU/ml of plasma on day 7]) (Table 1). Of the animals that survived following antibody treatment, in only one (from the 50-mg/kg treatment group) was virus undetectable in plasma at day 7, as determined by plaque assay. The remaining surviving animals had low to intermediate virus titers in serum at day 7 (Table 1). Treatment with the two highest doses of MAb KZ52 at the time of challenge, therefore, protected five of six animals from lethal infection but did not generally provide sterile protection, since the virus was detectable in the plasma in most (four of five) of these animals. Interestingly, three animals survived with significant plasma viremia, hinting that the benefit of antibody might extend beyond simple reduction of the viral load. For instance, antibody might mediate killing of infected cells that would not necessarily be immediately reflected by the magnitude of viremia.

Because MAb KZ52 did not provide sterile protection against Ebola virus challenge even when administered at a high

TABLE 1. Protection against Ebola Zaire virus challenge[a]

Antibody	Antibody dose (mg/kg)	Time of antibody treatment (h)	Day of death	Plasma viremia (log PFU/ml)[b]
KZ52	50	0	—	<1.4
	50	0	—	1.4
	50	0	—	4.5
	5	0	—	3.9
	5	0	—	3.9
	5	0	14	5.5
	0.5	0	7	4.7
	0.5	0	8	4.3
	0.5	0	7	5.1
Human IgG	50	0	7	4.2
	50	0	7	4.5
	50	0	7	5.6
	5	0	12	4.9
	5	0	12	4.2
	5	0	12	5.1
	0.5	0	9	4.8
	0.5	0	9	4.1
	0.5	0	7	3.9
None	n/a	n/a	9	4.1
	n/a	n/a	9	4.4
	n/a	n/a	10	2.8
	n/a	n/a	10	2.8
	n/a	n/a	—	2.6

[a] Animals were treated with the antibody and dose indicated at the time of challenge. —, animal lived; n/a, not applicable.
[b] Plasma viremia was assessed on day 7 postchallenge.

TABLE 2. Postexposure prophylaxis of Ebola Zaire virus challenge[a]

Antibody	Antibody dose[b]	Time of antibody treatment (h)	Day of death	Plasma viremia (log PFU/ml)[c]
KZ52	25	−1	—	<1.4
	25	−1	—	<1.4
	25	−1	—	<1.4
	25	−1	—	<1.4
	25	−1	—	<1.4
	25	+1	—	2.4
	25	+1	—	3.5
	25	+1	—	4.0
	25	+1	—	3.5
	25	+1	9	6.2
	25	+6	11	4.3
	25	+6	9	2.9
	25	+6	9	4.6
	25	+6	10	4.8
	25	+6	8	5.2
Equine IgG	3	0	—	<1.4
	3	0	—	<1.4
	3	0	—	<1.4
	3	0	—	<1.4
	3	0	—	<1.4
None	n/a	n/a	10	4.1
	n/a	n/a	9	4.3
	n/a	n/a	9	5.0
	n/a	n/a	8	4.9
	n/a	n/a	8	4.9

[a] Animals were administered the antibody at the indicated dose before or after challenge. —, animal lived; n/a, not applicable.
[b] KZ52 dose is in milligrams per kilogram of body weight; equine IgG dose is in milliliters per kilogram.
[c] Plasma viremia was assessed on day 7 postchallenge.

concentration (50 mg/kg; neutralization titer, ~1:200) at the time of challenge, we performed a preexposure prophylaxis experiment. We injected MAb KZ52 at 25 mg/kg and challenged with Ebola Zaire virus 1 h later. Four of five animals were completely protected from disease. One animal died, although there was a marked delay in the time of death (i.e., 15 days). Analysis of plasma on day 7 indicated that the protected animals did not have detectable virus (plasma viremia, <1.4 log PFU/ml), as determined by plaque assay, whereas a small amount of virus was detected in the plasma of the animal that died (plasma viremia, 2.7 log PFU/ml). However, protection was not sterile, since sera tested positive (>1:80) for guinea pig anti-Ebola virus antigen reactivity in an indirect fluorescent antibody test at 30 days postchallenge. The indirect fluorescent antibody test was performed as described previously (15, 17). Control tests performed with appropriate concentrations of purified MAb KZ52 were negative, indicating that this reactivity was not due to cross-reactivity of the goat anti-guinea pig IgG–fluorescein isothiocyanate conjugate with residual MAb KZ52 in the guinea pig sera. High titers of preexisting neutralizing antibody, therefore, effectively suppressed plasma viremia and provided protection from lethal infection but did not appear to provide sterile immunity in this model.

Next, we evaluated the ability of MAb KZ52 to provide postexposure protection against Ebola virus challenge. We injected a 25 mg/kg of KZ52 at −1 h, +1 h, and +6 h relative to the time of challenge with 10,000 PFU of guinea pig-adapted Ebola Zaire virus (Table 2). Preexposure prophylaxis at 1 h prior to virus challenge protected all five animals from lethal infection, in agreement with the results of the experiment described above. Significantly, postexposure prophylactic treatment with KZ52 at 1 h after virus challenge protected four of five animals from lethal infection. Protection was not sterile, since plasma viremia (ranging from 2.4 to 4.0 log PFU) was detected in all surviving animals. The animal that died had a very high plasma viremia (6.2 log PFU). No protection was observed after postexposure prophylaxis with KZ52 at 6 h after virus challenge.

As an additional control, we treated animals with 3 ml of neutralizing equine IgG/kg at the time of challenge. The equine IgG against Ebola virus (18) was obtained from the World Health Organization, which obtained the material from the Russian association Epidbiomed, and has been described in detail in elsewhere (16, 17). This treatment has previously been shown to provide protection against infection in this model (17). As expected, all animals were protected and no virus was detected in their plasma. In concordance with the experiments with KZ52, all animals treated with the neutralizing equine IgG seroconverted (as assessed on day 30 after challenge), suggesting that this treatment also did not appear to provide sterile protection against Ebola Zaire virus in this animal model. Untreated animals all experienced high plasma viremia and died between days 8 and 10. As in the experiment described above, a number of antibody-treated animals protected in this study developed significant viral loads, again hinting that antibody action may extend beyond controlling viremia.

Our experiments show that a neutralizing human antibody against Ebola virus derived from a natural infection can provide protection against robust virus challenge in an animal model. Protection against lethal infection with this relatively

high dose of virus was achieved even if the antibody was given up to 1 h postchallenge. The dose of virus used in our study, 10,000 PFU, may seem high, but it was chosen to be representative of the very high levels of viremia that filoviruses can achieve in bodily fluids of infected individuals in a relatively short period of time. For example, Ebola virus has been shown to replicate to levels of $10^{6.5}$ PFU/ml of blood in humans (1) and to 10^7 to 10^8 PFU/ml of blood in monkeys (17), and bodily secretions can also contain high concentrations of virus. For example, it has been shown that another filovirus, Marburg virus, is excreted at high levels (up to 10^6 guinea pig infectious doses) in urine (33). The challenge dose of 10,000 PFU employed in our study, therefore, corresponds to the amount of Ebola virus contained in 1 to 10 μl of blood or bodily fluid from an acutely ill individual. Several recent studies have used smaller challenge doses (35, 36). Presently, it is unclear whether the amount of antibody required to protect against a high-dose challenge differs from that required to protect against a low-dose challenge. From a thermodynamic perspective, the virus dose should be irrelevant because antibodies are typically in vast molar excesses compared to virus (e.g., $>10^{14}$ in our experiment, assuming an initial virus concentration of 10,000 particles/ml). It should be noted, however, that this does not take into account considerations such as target cell availability and the presence of physical barriers to virus entry. Higher challenge doses, therefore, will have a statistical advantage in establishing a productive infection. Since Ebola virus concentrations in biological fluids are high, it therefore seems prudent to challenge with relatively high doses of virus.

Our experiments indicate that MAb KZ52 can provide protection against lethal Ebola virus infection by reducing plasma viremia, since many protected animals had very low levels of viremia. Interestingly, it has been determined that in the case of human infection, persons who are acutely ill are typically intensely viremic. Most infected persons die at this time (30). The probability of survival, however, increases dramatically for persons that survive at least 1 week after the onset of symptoms, and survivors then recover relatively rapidly and completely (30, 31). Postexposure prophylaxis with KZ52 at 1 h postchallenge resulted in an order of magnitude reduction of plasma viremia (mean of 3.6 log PFU in surviving animals, compared to a mean of 4.8 log PFU in the control group) (Table 2). This reduction in viremia may have enabled the animals to survive the initial course of the infection. Postexposure prophylaxis at 6 h after challenge did not affect plasma viremia and also did not protect against lethal infection.

Although a reduction in plasma viremia is associated with increased survival following Ebola virus challenge, our results also indicate that some antibody-treated animals survive (Table 1, 5 mg/kg-treated animals; Table 2, + 1 h-treated animals) with significant viremias that overlap those observed in untreated animals that succumb to infection. This hints at an action of antibody that extends beyond a reduction in viremia by virus neutralization. One possibility, for example, is that the antibody acts against infected cells, helping to control critical tissue damage. This is undoubtedly an area to consider for further investigation.

The results with the neutralizing human antibody KZ52 are very encouraging. This is the first time that an antibody has been shown to be efficacious in postexposure prophylaxis of Ebola virus infection in guinea pigs. Thus far, only the neutralizing equine IgG has shown comparable neutralizing ability. The equine IgG was not able to protect monkeys from Ebola virus challenge, although it was effective in the guinea pig model. The predictive value of the guinea pig model for protection against infection in primates has therefore been questioned. A caveat to the equine IgG studies should be noted, however. The neutralizing equine IgG was prepared by immunizing horses with the homogenized livers of Ebola virus-infected monkeys and therefore contains antibodies against monkey cells. The Ebola challenge viruses used in guinea pig and mouse studies have typically been grown in Vero cells (derived from African green monkey kidneys) (5, 11). Therefore, the possibility that protection in the guinea pig model was provided in part by antibodies against monkey cell antigens carried on the Ebola virus envelope, as has been described for simian immunodeficiency virus (9, 34), cannot be excluded. This protective ability might not be apparent in monkeys, in which the anti-monkey cell activity would be rapidly adsorbed by the host.

Future studies will address the efficacy of MAb KZ52 in protecting nonhuman primates against Ebola virus challenge. If effective, KZ52 will be a promising candidate for development as an urgent immunoprophylactic agent to prevent Ebola virus infection in humans. It may be particularly useful for the preexposure prophylaxis of Ebola virus infection in workers sent to aid in an Ebola virus outbreak or bioterrorist attack.

We are grateful to Ann Hessell and Paul Carney for antibody production and to Joan Geisbert for technical assistance in the BSL4 facility. We thank Igor Kozlov and Natalie Prigozhina for translating Russian language publications.

This work was supported in part by NIH grant AI48053 (to D.R.B.).

REFERENCES

1. **Anonymous.** 1978. Ebola haemorrhagic fever in Zaire, 1976. Bull. W. H. O. **56**:271–293.
2. **Baba, T. W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L. A. Cavacini, M. R. Posner, H. Katinger, G. Stiegler, B. J. Bernacky, T. A. Rizvi, R. Schmidt, L. R. Hill, M. E. Keeling, Y. Lu, J. E. Wright, T. C. Chou, and R. M. Ruprecht.** 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat. Med. **6:**200–206.
3. **Borisevich, I. V., V. V. Mikhailov, V. P. Krasnyanskii, V. N. Gradoboev, E. V. Lebedinskaia, N. V. Potryvaeva, and G. D. Timan'kova.** 1995. Development and study of the properties of immunoglobulin against Ebola fever. Vopr. Virusol. **40:**270–273.
4. **Bowen, E. T. W., G. Lloyd, G. Platt, L. B. McArdell, P. A. Webb, and D. I. H. Simpson.** 1978. Virological studies on a case of Ebola virus infection in man and in monkeys, p. 80–85. *In* S. R. Pattyn (ed.), Ebola virus haemorrhagic fever. Elsevier, Amsterdam, The Netherlands.
5. **Bray, M., K. Davis, T. Geisbert, C. Schmaljohn, and J. Huggins.** 1999. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J. Infect. Dis. **179**(Suppl. 1)**:**S248–S258.
6. **Burton, D. R., and P. W. H. I. Parren.** 2000. Fighting the Ebola virus. Nature **408:**527–528.
7. **Burton, D. R., and P. W. H. I. Parren.** 2000. Vaccines and the induction of functional antibodies: time to look beyond the molecules of natural infection? Nat. Med. **6:**123–125.
8. **Casadevall, A., and M. D. Scharff.** 1995. Return to the past: the case for antibody-based therapies in infectious diseases. Clin. Infect. Dis. **21:**150–161.
9. **Chan, W. L., A. Rogers, R. D. Hancock, F. Taffs, P. Kitchin, G. Farrar, and F. Y. Liew.** 1992. Protection in simian immunodeficiency virus-vaccinated monkeys correlates with anti-HLA class I antibody response. J. Exp. Med. **176:**1203–1207.
10. **Chanock, R. M., J. E. Crowe, Jr., B. R. Murphy, and D. R. Burton.** 1993. Human monoclonal antibody Fab fragments cloned from combinatorial libraries: potential usefulness in prevention and/or treatment of major human viral diseases. Infect. Agents Dis. **2:**118–131.
11. **Connolly, B. M., K. E. Steele, K. J. Davis, T. W. Geisbert, W. M. Kell, N. K.**

Jaax, and P. B. Jahrling. 1999. Pathogenesis of experimental Ebola virus infection in guinea pigs. J. Infect. Dis. **179**(Suppl. 1)**:**S203–S217.
12. **Emond, R. T. D., B. Evans, E. T. W. Bowen, and G. Lloyd.** 1977. A case of Ebola virus infection. Br. Med. J. **2:**541–544.
13. **Gauduin, M. C., P. W. H. I. Parren, R. Weir, C. F. Barbas III, D. R. Burton, and R. A. Koup.** 1997. Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat. Med. **3:**1389–1393.
14. **Hevey, M., D. Negley, J. Geisbert, P. B. Jahrling, and A. Schmaljohn.** 1997. Antigenicity and vaccine potential of Marburg virus glycoprotein expressed by baculovirus recombinants. Virology **239:**206–216.
15. **Jahrling, P. B.** 1995. Filoviruses and arenaviruses, p. 1068–1081. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
16. **Jahrling, P. B., J. Geisbert, J. R. Swearengen, G. P. Jaax, T. Lewis, J. W. Huggins, J. J. Schmidt, J. W. LeDue, and C. J. Peters.** 1996. Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. Arch. Virol. Suppl. **11:**135–140.
17. **Jahrling, P. B., T. W. Geisbert, J. B. Geisbert, J. R. Swearengen, M. Bray, N. K. Jaax, J. W. Huggins, J. W. LeDuc, and C. J. Peters.** 1999. Evaluation of immune globulin and recombinant interferon-α2b for treatment of experimental Ebola virus infections. J. Infect. Dis. **179**(Suppl. 1)**:**S224–S234.
18. **Krasnyanskii, V. P., V. V. Mikhailov, I. V. Borisevich, V. N. Gradoboev, A. A. Evseev, and V. A. Pshenichnov.** 1994. Preparation of hyperimmune horse serum to Ebola virus. Vopr. Virusol. **39:**91–92.
19. **Ksiazek, T. G.** 1991. Laboratory diagnosis of filovirus infections in nonhuman primates. Lab. Anim. **20:**34–46.
20. **Kudoyarova-Zubavichene, N. M., N. N. Sergeyev, A. A. Chepurnov, and S. V. Netesov.** 1999. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. J. Infect. Dis. **179**(Suppl. 1)**:**S218–S223.
21. **Markin, V. A., V. V. Mikhailov, V. P. Krasnyansky, I. V. Borisevich, and I. V. Firsova.** 1997. Development of methods for urgent prophylaxis and treatment of Ebola fever. Vopr. Virusol. **42:**31–33.
22. **Maruyama, T., P. W. H. I. Parren, A. Sanchez, I. Rensink, L. L. Rodriguez, A. Khan, C. J. Peters, and D. R. Burton.** 1999. Recombinant human monoclonal antibodies to Ebola virus. J. Infect. Dis. **179**(Suppl. 1)**:**S235–S239.
23. **Maruyama, T., L. L. Rodriguez, P. B. Jahrling, A. Sanchez, A. S. Khan, S. T. Nichol, C. J. Peters, P. W. H. I. Parren, and D. R. Burton.** 1999. Ebola virus can be effectively neutralized by antibody produced in natural human infection. J. Virol. **73:**6024–6030.
24. **Mascola, J. R., M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. Brown, C. V. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, H. Katinger, and D. L. Birx.** 1999. Protection of macaques against pathogenic SHIV-89.6PD by passive transfer of neutralizing antibodies. J. Virol. **73:** 4009–4018.
25. **Mascola, J. R., G. Stiegler, T. C. VanCott, H. Katinger, C. B. Carpenter, C. E. Hanson, H. Beary, D. Hayes, S. S. Frankel, D. L. Birx, and M. G. Lewis.** 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. **6:**207–210.
26. **McCormick, J. B., S. P. Bauer, L. H. Elliott, P. A. Webb, and K. M. Johnson.** 1983. Biologic differences between strains of Ebola virus from Zaire and Sudan. J. Infect. Dis. **147:**264–267.
27. **Mupapa, K., M. Massamba, K. Kibadi, K. Kumula, A. Bwaka, M. Kipasa, R. Colebunders, J. J. Muyembe-Tamfum, and the International Scientific and Technical Committee.** 1999. Treatment of Ebola haemorrhagic fever with blood transfusions from convalescent patients. J. Infect. Dis. **179**(Suppl. 1)**:**S18–S23.

27a. **National Research Council.** 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.

28. **Parren, P. W. H. I., and D. R. Burton.** 2001. The anti-viral activity of antibodies in vitro and in vivo. Adv. Immunol. **77:**195–262.
29. **Parren, P. W. H. I., P. A. Marx, A. J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, and D. R. Burton.** 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J. Virol. **75:**8340–8347.
30. **Peters, C. J., and J. W. LeDuc.** 1999. An introduction to Ebola: the virus and the disease. J. Infect. Dis. **179**(Suppl. 1)**:**ix–xvi.
31. **Sadek, R. F., A. S. Khan, G. Stevens, C. J. Peters, and T. G. Ksiazek.** 1999. Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995: determinants of survival. J. Infect. Dis. **179**(Suppl. 1)**:**S24–S27.
32. **Siegert, R., H. L. Shu, and W. Slenczka.** 1968. Isolierung und Identifizierung des "Marburg-Virus." Dtsch. Med. Wochenschr. **93:**604–612.
33. **Simpson, D. I. H.** 1969. Marburg virus disease: experimental infection of monkeys. Lab. Anim. Handb. **4:**149–154.
34. **Stott, E. J.** 1994. Towards a vaccine against AIDS: lessons from simian immunodeficiency virus vaccines. Curr. Top. Microbiol. Immunol. **188:**221–237.
35. **Sullivan, N. J., A. Sanchez, P. E. Rollin, Z. Y. Yang, and G. J. Nabel.** 2000. Development of a preventive vaccine for Ebola virus infection in primates. Nature **408:**605–609.
36. **Vanderzanden, L., M. Bray, D. Fuller, T. Roberts, D. Custer, K. Spik, P. B. Jahrling, J. Huggins, A. Schmaljohn, and C. Schmaljohn.** 1998. DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. Virology **246:**134–144.
37. **Wilson, J. A., M. Hevey, R. Bakken, S. Guest, M. Bray, A. L. Schmaljohn, and M. K. Hart.** 2000. Epitopes involved in antibody-mediated protection from Ebola virus. Science **287:**1664–1666.